TECHNICAL NOTE

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The Analysis of Soil Samples by Reverse Phase-High Performance Liquid Chromatography Using Wavelength Ratioing

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ABSTRACT: The technique of reverse phase high performance liquid chromatography (HPLC) is investigated for the forensic analysis of soils. Unlike in previous works on this subject, the method of detection of the peaks is modified to include detection at two different wavelengths in the ultraviolet and the ratio of the absorption intensities is determined and displayed. The results show that the chromatograms of all of the soil samples studied differ from each other quantitatively but not all can be differentiated qualitatively. It is concluded that this method of analysis is an excellent presumptive test but has not been proven to be individualizing. To increase the probative value of the test, another method of separation and perhaps detection must be chosen.

KEYWORDS: forensic science, soils, chromatographic analysis, soil analysis, high performance liquid chromatography

Traditionally, soil has been considered to be class evidence (as opposed to individual evidence) in forensic science cases. As such it can contribute to the evidence that places a person at a crime scene. The potential of soil as evidence lies in the almost infinite variety of soils and the fact that soil can change greatly over even short distances—horizontally and vertically.

Although there have been cases where a soil sample has contained some foreign material such as glass or broken concrete which may make this soil truly unique, the typical forensic science case involves the determination of the probability that two soil samples could have had a common source. From a statistical standpoint this is not possible and likely never will be because the number of different types of soil on earth and the precise extent to which they change from place to place are not known. In light of this, soil analysts have continually searched for methods of analysis that increase the degree of certainty with which soil comparisons are made even if no mathematical probabilities can be assigned to the conclusions [*1*].

Traditional methods of forensic soil analysis have been largely presumptive in nature; they seek to compare general class characteristics of soil samples and thus, such evidence can only

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be compared with a low degree of certainty. Such examinations include microscopic examination for mineral content [2,3] which requires a great deal of skill for proper interpretation, density gradient analysis [4,5], and the determination of color and particle size distribution [6].

In recent years, methods of soil analysis have become more instrument-oriented to determine the types and methods of specific organic or minerological constituents. These methods include neutron activation analysis, X-ray fluorescence, and emission and absorption spectrometry [7]. Also, measurements of enzyme activity [8] and pH [9] have been attempted but without much benefit to forensic science cases.

Various chromatographic techniques have been employed for some time in the analysis of a large variety of forensic science evidence including soil. Most of the chromatographic analysis of soils has recently focused upon reverse phase high performance liquid chromatography (HPLC) [10-12]. These studies have employed simple organic extractions and HPLC using C_{18} reverse phase columns and isocratic elution using various combinations of methanol, acetonitrile, and water as the mobile phase. Detection has been exclusively by ultraviolet radiation (UV) at 254 nm.

The purpose of this study was to determine if the method of detection of the HPLC peaks could be improved by yielding more information about the substances being eluted. This was accomplished by taking advantage of some of the special features of the instrument used in the study. The instrument was capable of scanning the UV spectrum of a peak as it eluted and then storing the wavelength of maximum absorption and its intensity. From this data a second wavelength of high intensity of absorbance could be chosen to monitor the elution. In addition, the instrument was capable of determining the ratio of the absorbances of the various eluents at two preselected wavelengths and display the ratio. The increased spectral information results in chromatograms that are easier to compare and contrast than those which result from monitoring absorption at a single wavelength.

Experimental Procedure

Equipment

The high performance liquid chromatograph was a Beckman Model 341 equipped with a Model 112 pump and Model 165 variable wavelength detector. The column was a Beckman Ultrasphere-ODS, 4.6 by 150 mm protected by a Beckman guard column containing the same packing. The UV detector was operated at 254 and 280 nm and in the ratio mode at 254/280. The recorder was a Kipp and Zonen dual channel Model 100. The soil samples were prefiltered with a Waters Associates Sample Clarification Kit.

Reagents

The acetonitrile and water used for the extractions and as the mobile phase were both Burdick-Jackson HPLC grade. The soil samples were obtained from the locations listed in Table 1. Approximately 50 g of surface soil were collected at each site.

Procedure

The extraction procedure used is essentially a modification of that suggested by Reuland and Trinler [12].

Each soil sample was placed in a petri dish and dried at 60°C overnight. A 5-g sample of each soil was extracted in 25 mL of acetonitrile at room temperature in an ultrasonic mixer for 15 min. The samples were then filtered once by suction in a filter flask to remove gross particulate matter. A second filtration was then done using the Sample Clarification Kit. The

TABLE 1—*Soil sampling sites.*[a]

1.	Creek edge
2.	City park
3.	City lawn
4.	River edge
5.	Wooded area
6.	Lake front
7.	Industrial area near cement plant
8.	Shopping center
9.	Industrial area near wood processing plant
10.	Suburban lawn
11.	Cultivated field

[a]All of the sites were within the city limits of Alpena, MI, a city of radius of 12 km (7.5 miles). There were eleven in all.

samples were then evaporated to a volume of 1 mL. Injection volumes of 20 μL were used throughout.

The HPLC was run isocratically using acetonitrile:water (90:10) as the mobile phase. The flow rate was set at 1 mL per minute and the chart speed at 10 mm per minute. There were three channels of output from the HPLC to the chart recorder. Channel 1 was set at 254 nm. Each sample was chromatographed at this wavelength and the UV spectrum of each of the major peaks was scanned on the run. The results of these scans indicated that many of the prevalent components of these soils had a lambda maximum of around 280 nm and this was chosen as the wavelength for Channel 2. Channel 3 acted as the bipolar analog output for the absorbance ratio of Channel 1 to Channel 2.

Results and Discussion

Figures 1 through 11 are the chromatograms of the soil samples monitored at 254 nm. Figures 12 through 22 are the corresponding samples obtained at 280 nm. Finally, Figs. 23 through 33 are the samples run using the ratio of the absorbances at 254/280.

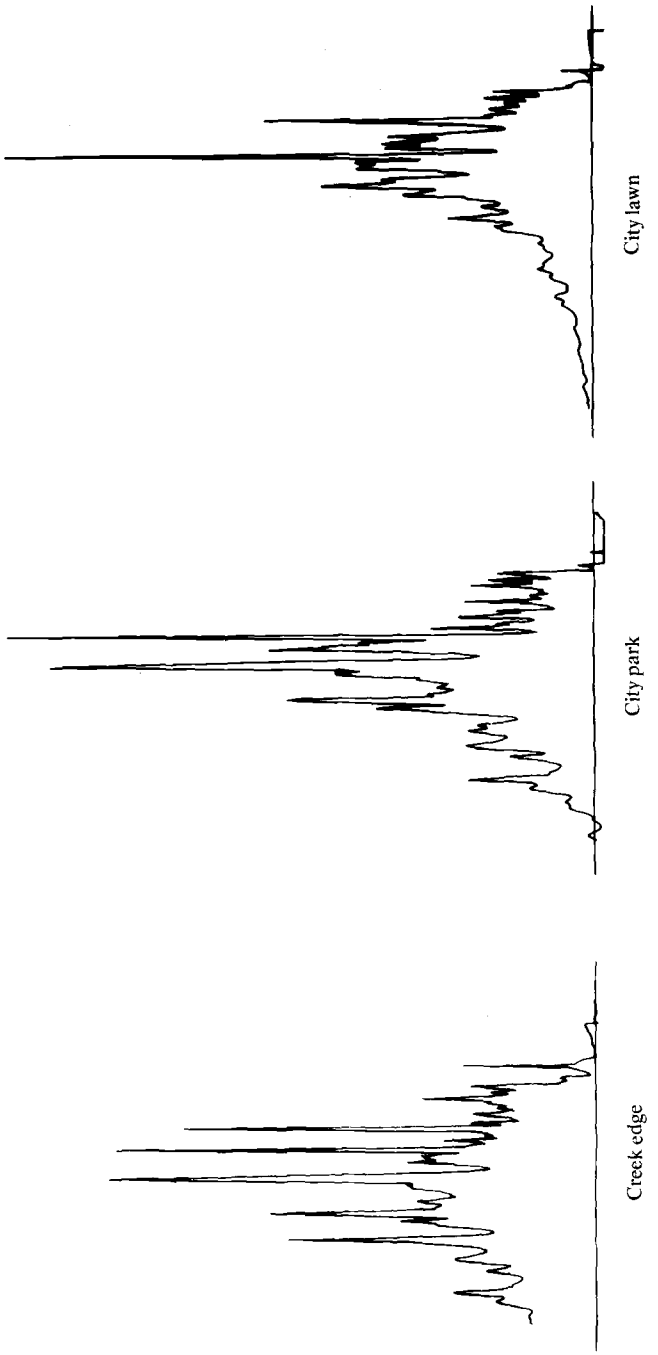
All of the chromatograms that were detected at 254 nm were compared with each other to see if they differed qualitatively or quantitatively or both. There were a total of 55 comparisons. The same was done for the samples detected at 280 nm and for the ratio chromatograms. Two samples were determined to differ qualitatively if at least one of the major peaks in one of the samples was totally absent in the other. If two chromatograms differed only in the proportional intensities of the major peaks then they were determined to differ quantitatively.

In the soil samples studied, all of the soils differed quantitatively no matter how detected but the same is not true qualitatively. Table 2 is a compilation of the results of the qualitative comparisons of all eleven soil samples. The numbers of the samples are keyed to the sites in Table 1. Examination of this table indicates that, of the 55 comparisons made of the samples at 254 nm, 21 could be differentiated qualitatively. Likewise, 19 of the 55 at 280 nm were qualitatively different and 23 of the 55 of the ratios differed in this manner.

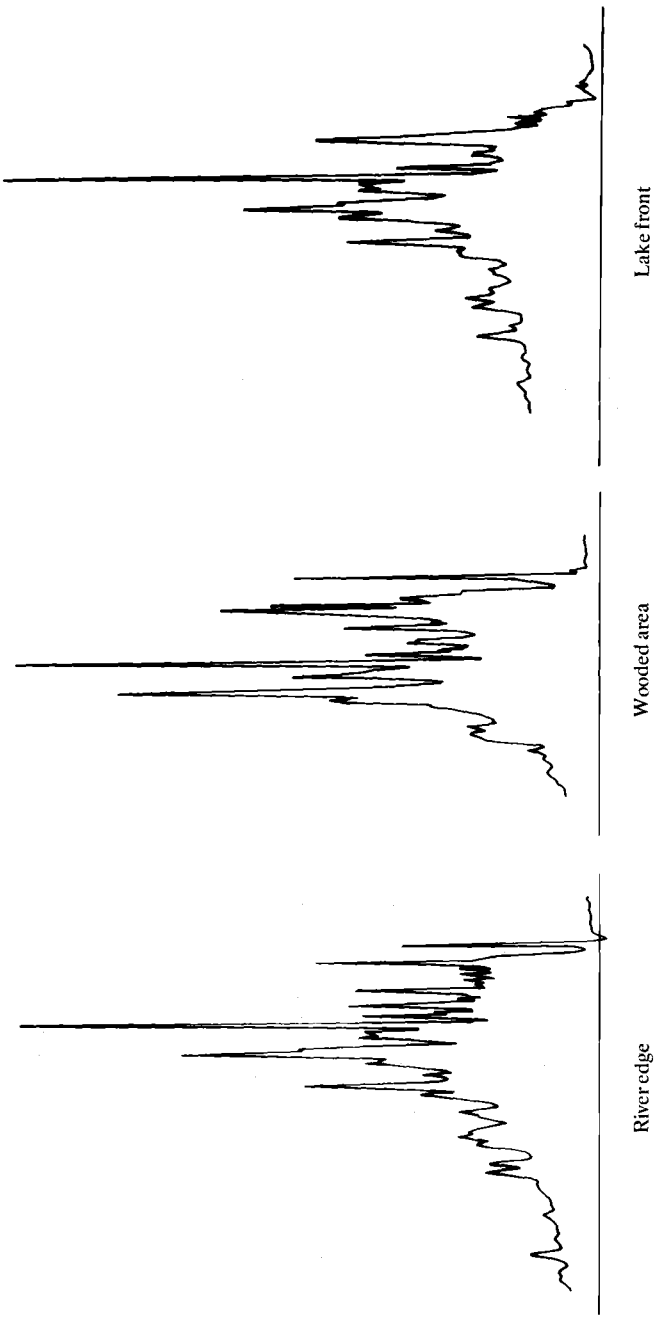
Six of the samples were qualitatively differentiated only at 254 nm whereas four were differentiated only at 280 nm and six by the intensity ratios. Eight samples could be qualitatively differentiated by all of the detection methods.

When the results of this study as well as those of the other HPLC studies cited are considered in total, there are several conclusions that are indicated:

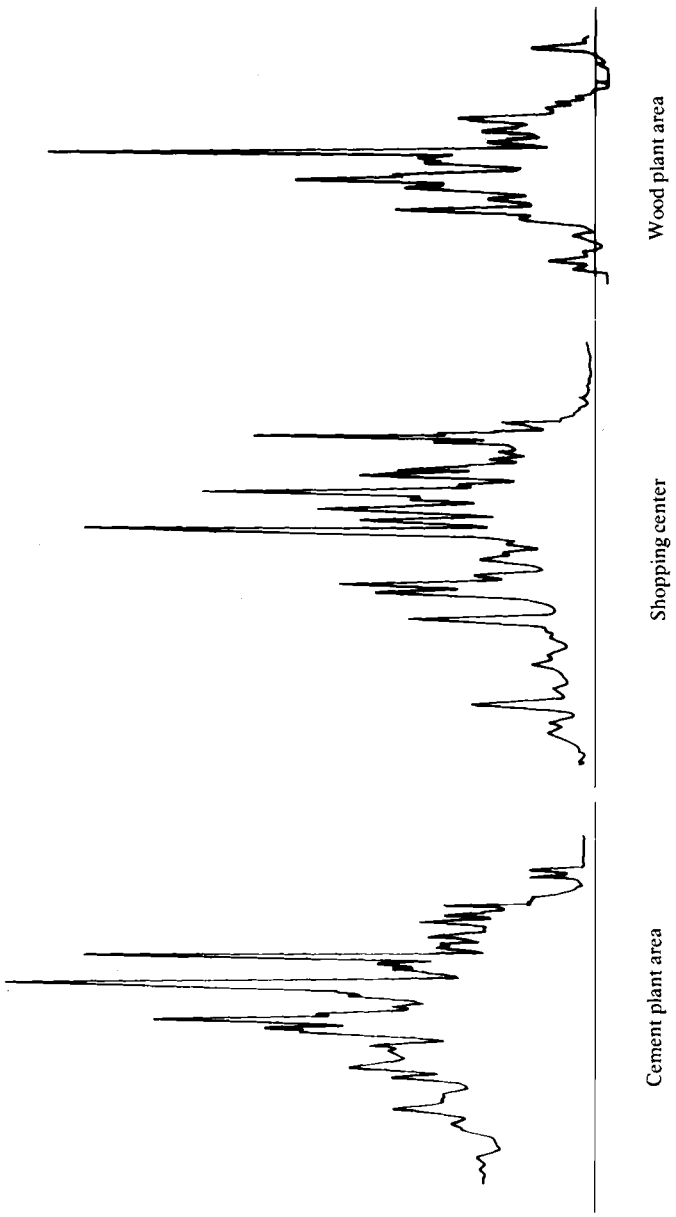
1. All of the soil samples evaluated thus far have been differentiated quantitatively by the method of reverse phase HPLC under the conditions of these studies. In addition, a large per-



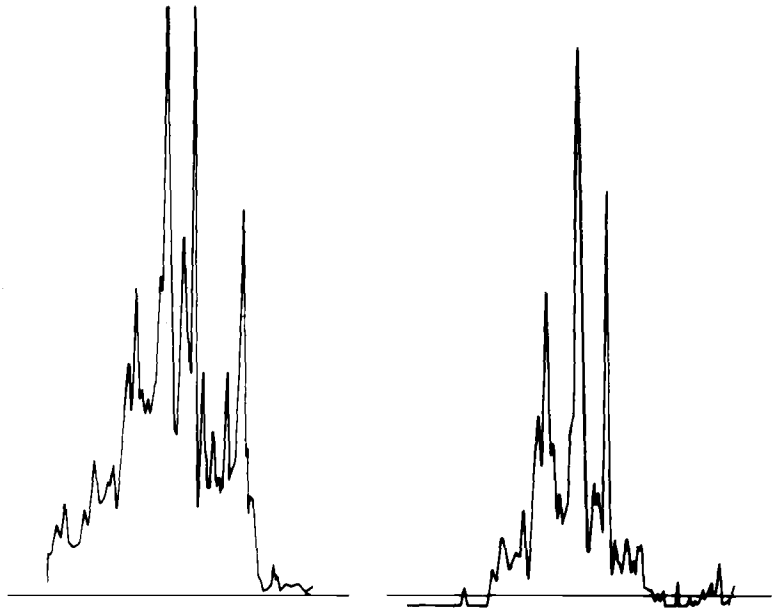
FIGS. 1-3—*Chromatograms of soil samples with detector set at 254 nm.*



FIGS. 4-6—*Chromatograms of soil samples with detector set at 254 nm.*



FIGS. 7-9—*Chromatograms of soil samples with detector set at 254 nm.*

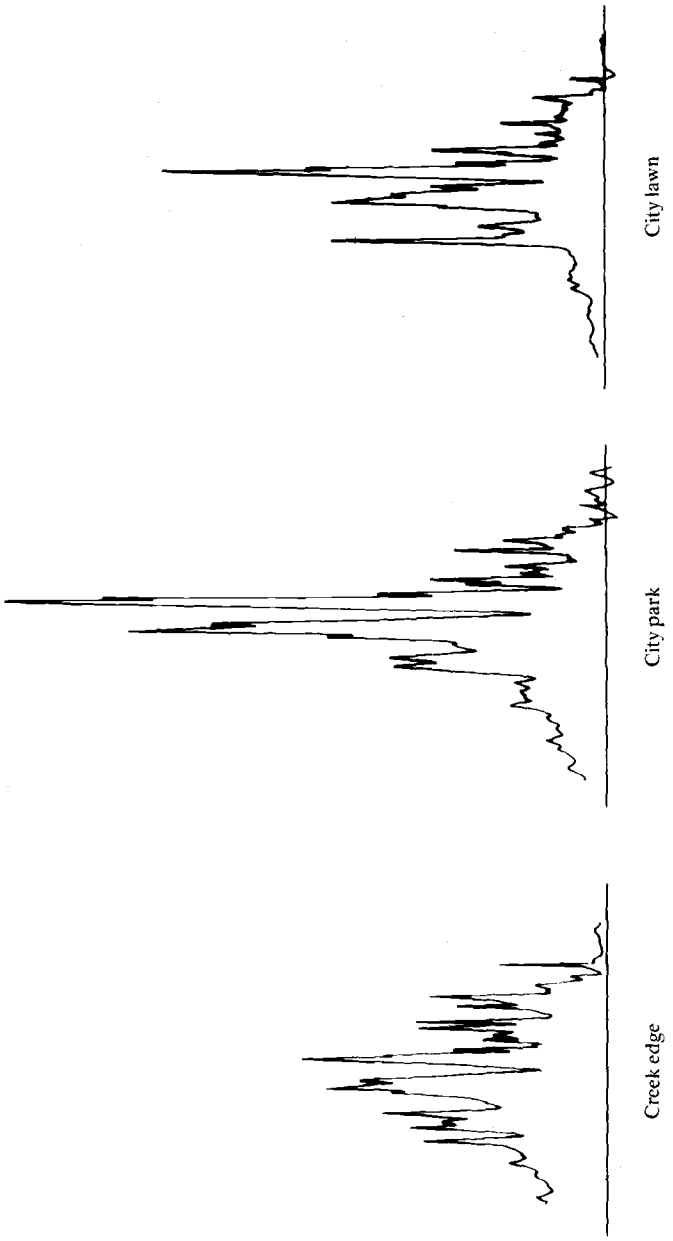


Suburban lawn Cultivated field

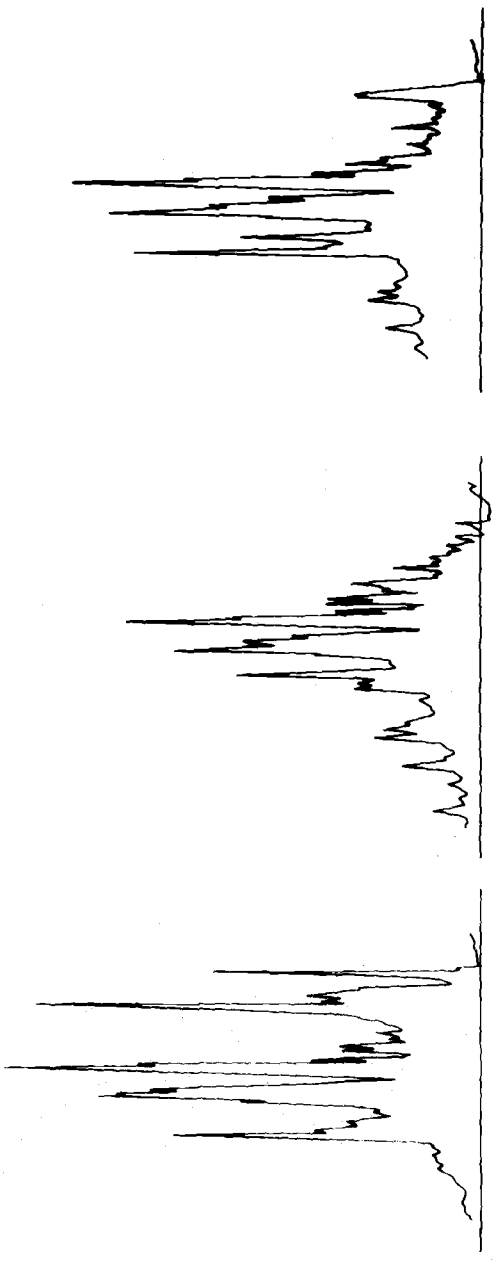
FIGS. 10-11—*Chromatograms of soil samples with detector set at 254 nm.*

TABLE 2—*Comparison of soil chromatograms for qualitative differences.*

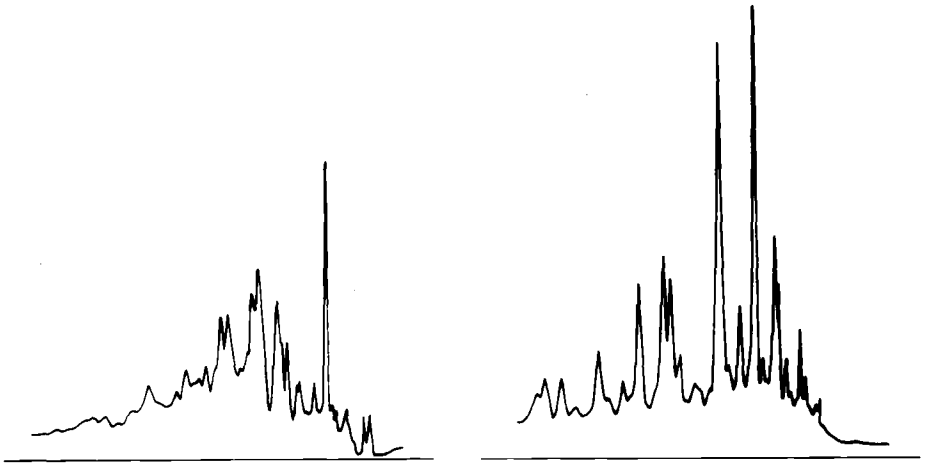
Sample	1	2	3	4	5	6	7	8	9	10	11
1		254	254		254	354		254	254		
							280				280
			ratio				ratio				
2								254		254	
							280				
			ratio	ratio				ratio			
3					254		254			254	254
							280	280			280
				ratio	ratio		ratio	ratio	ratio		ratio
4								254			
					280			280			
								ratio			ratio
5								254	254	254	
							280	280	280	280	
							ratio	ratio			
6							254			254	
							280	280		280	
							ratio	ratio	ratio	ratio	
7								254	254		
								280			
								ratio	ratio		
8										254	
									280	280	280
									ratio	ratio	ratio
9											
10											
11											



FIGS. 12-14—*Chromatograms of soil samples with detector set at 280 nm.*



FIGS. 15-17—*Chromatograms of soil samples with detector set at 280 nm.*



Cement plant area

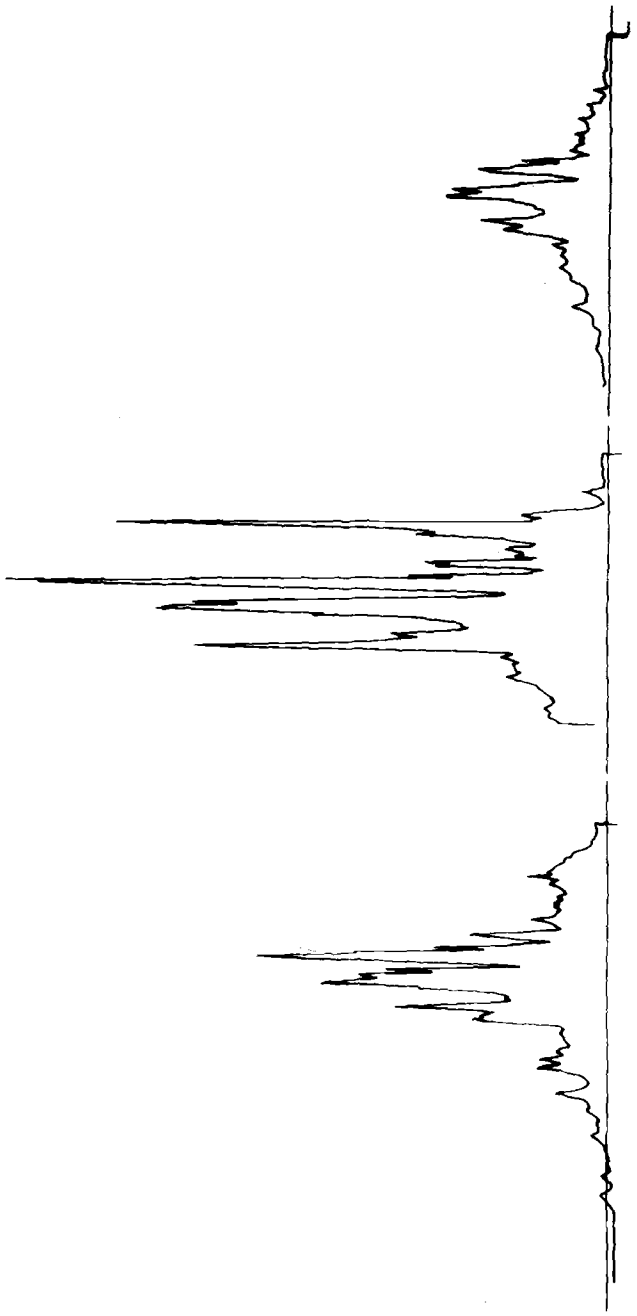
Shopping center

FIGS. 18-19—*Chromatograms of soil samples with detector set at 280 nm.*

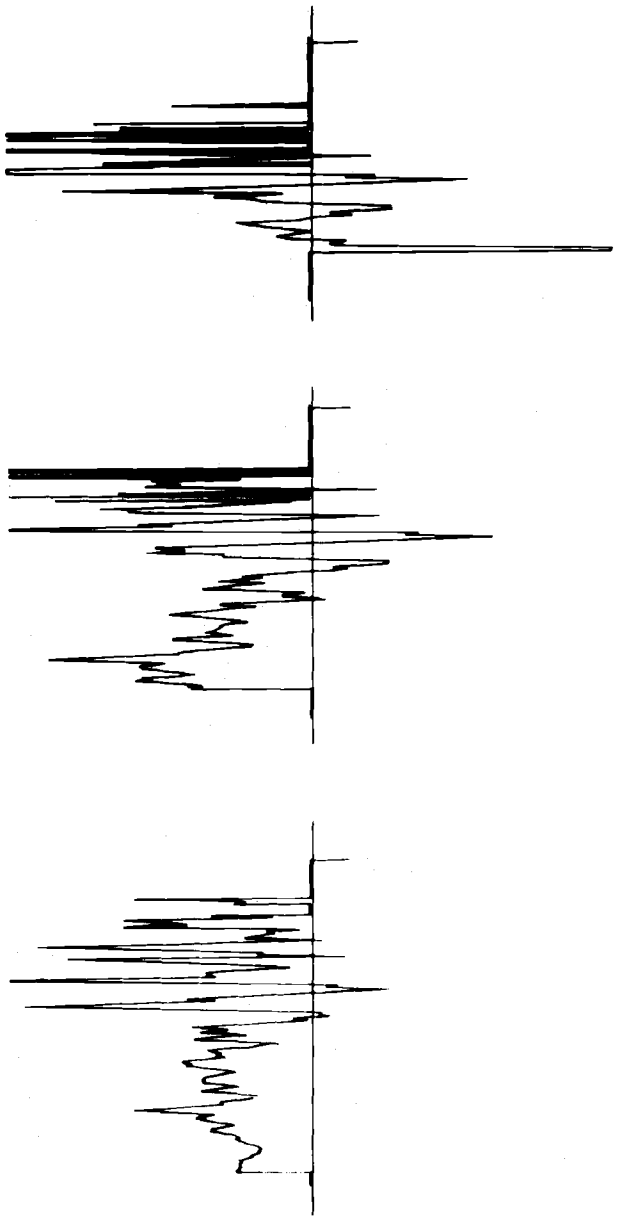
centage of the samples can be differentiated qualitatively as well. As such, this method is an excellent presumptive test for soils in forensic science cases especially when used with other analytical results. However, the test cannot be said to be individualizing on the basis of these limited studies since that would only be possible if all samples, even those taken within a short distance of each other vertically or horizontally could be unequivocally differentiated qualitatively.

2. It was originally thought that the ability to monitor the components of the soil in the HPLC analysis at more than one wavelength, especially if the intensity ratios of two wavelengths could be displayed, might reveal enough additional information about the soil samples that they could perhaps be individualized. The results of this study do not bear out this hypothesis. Indeed, this method provides essentially no more discriminating power than does detection at 254 nm by itself. In retrospect, this is not that surprising because the same components are evidently being detected at either wavelength albeit at different intensities.

3. If HPLC is to be of more probative value as a test in soils, there will, in all likelihood, have to be changes in the way that the soils are separated and detected. With respect to separation, the literature indicates that some form of size exclusion HPLC can be used to characterize effectively soil samples for purposes other than as evidence in soils such as to monitor pesticides, fertilizers, and so forth [*13,14*]. This method is presently undergoing evaluation in this laboratory. As for methods of detection, the nature of some of the components of soil indicates that perhaps fluorescence detection might show promise. This hypothesis is also being tested in this laboratory.

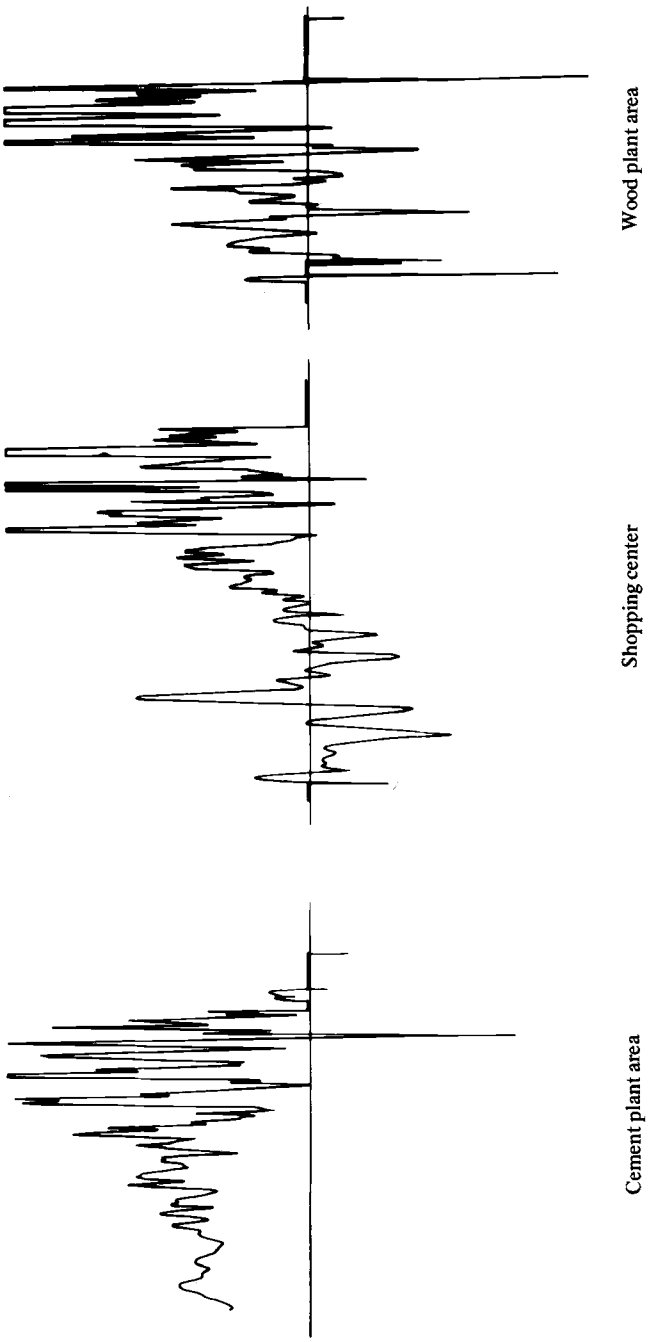


FIGS. 20-22.—*Chromatograms of soil samples with detector set at 280 nm.*

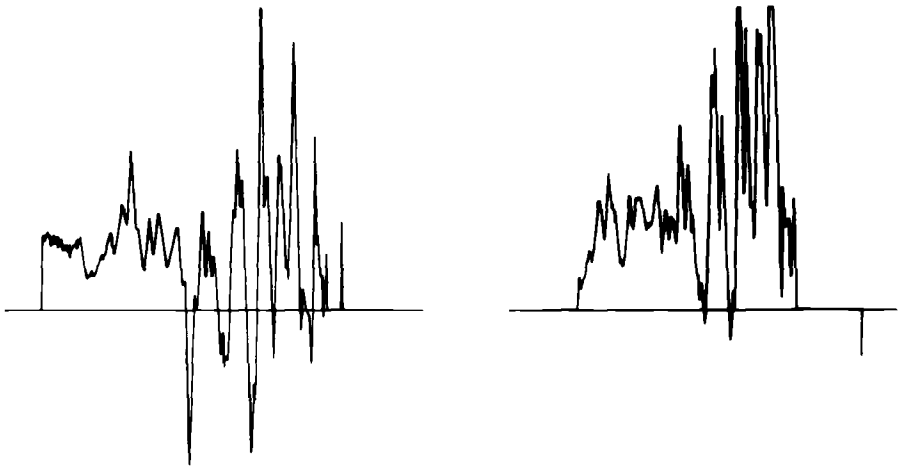


FIGS. 23–25—*Chromatograms of soil samples with detector set at intensity ratios of 254/280 nm.*

FIGS. 26-28—*Chromatograms of soil samples with detector set at intensity ratios of 254/280 nm.*



FIGS. 29–31—*Chromatograms of soil samples with detector set at intensity ratios of 254/280 nm.*



Cultivated field

Suburban lawn

FIGS. 32-33—*Chromatograms of soil samples with detector set at intensity ratios of 254/280 nm.*

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